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13017

Prothrombin Level of Blood After the Intramuscular Injection of Sodium Citrate.*

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Shafiroff, Doubilet and Co Tui[1] have reported that the "prothrombin time" according to my method is decreased in dogs following the intramuscular injection of sodium citrate. Theoretically the "prothrombin time" can be altered by a change in the convertibility of prothrombin, by a further increase in thromboplastin, or by an actual alteration in the prothrombin level. A change in the convertibility of prothrombin remains a hypothetical conjecture which has never been satisfactorily demonstrated experimentally, although claimed by some investigators.[2] The decrease in "prothrombin

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1 Shafiroff, B. G. P., Doubilet, H., and Co Tui, PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 136.

2 Brinkhous, K. M., *Medicine*, 1940, **19**, 329.

time" may well be due to a liberation of thromboplastin from the platelets, but a change in the clotting time under these conditions can occur only if the thromboplastin employed in the test does not possess maximum potency. By using a thromboplastin having a high degree of activity, a minimum fixed clotting time is obtained, which cannot be further reduced by additional amounts of this activating factor. The most satisfactory preparation now known is rabbit brain dehydrated with acetone.[3] By means of this product, human plasma is clotted in 11 to 12½ seconds, and rabbit and dog plasma in 5½ to 6½ seconds.

Experimental. Dogs and rabbits were given sodium citrate intramuscularly and the prothrombin of the blood determined at regular intervals following the injection. Since the clotting time (prothrombin time) of undiluted plasma is very rapid, the test was also made on diluted plasma in which alterations in the prothrombin concentration cause relatively greater changes in the clotting time.

TABLE I.

Effect of Sodium Citrate (Intramuscularly)* on Prothrombin Level of the Blood.

	Body wt kg	Clotting time (in seconds) of recalcified oxalated plasma with excess thromboplastin: Before injection	30 min.	60 min.	90 min.	120 min.	
Dog A	12	6.5	6.5	6.5	6.5	6.5	
		12	12	11.5	11	11	Plasma diluted with 4 vol. saline sol.
" B	7	6.5	6.5	6.5	6.5	6	
		11.5	12.5	12.5	11.5	10	Plasma diluted as above
Rabbit A	2.9	5.5	5.5	5.5	5.5	5.5	
		11.5	11.5	12	12		" " " "
" B	2.1	5.5	5.5	5.5	5.5		
		11.5	11	11.5	11.5		" " " "

*Each animal received 0.5 g of sodium citrate (10% solution) per kg intramuscularly.

The prothrombin was determined by the author's method.[4] The thromboplastin used was made February 26, 1940, and preserved in an evacuated glass tube.

Results. The data recorded in Table I clearly demonstrate that the injection of sodium citrate did not alter the prothrombin level of the blood. The decrease in clotting time that is observed after the administration of sodium citrate as well as the apparent change of "prothrombin time" obtained by Shafiroff, *et al.,* is not due to a change in the prothrombin, but is brought about by some other

[3] Quick, A. J., *Science,* 1940, **92**, 113.

[4] Quick, A. J., *J. Am. Med. Assn.,* 1938, **110**, 1658.

factor. In view of the fact that a thromboplastin with high and constant activity can readily be prepared, there is no longer any valid justification for attempting quantitative studies of prothrombin with preparations of variable and uncertain potency.

Summary. The intramuscular injection of sodium citrate in dogs and rabbits does not change the prothrombin level of the blood.

13018

Determination of Pantothenic Acid in Normal Blood and Urine by Microbiological Technic.

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In an earlier report from this laboratory[1] a technic was described for the assay of pantothenic acid based upon the growth response of the bacterium *Proteus morganii* to the vitamin. The application of this technic to the assay of pantothenic acid in natural substances, particularly blood and urine, is described in this communication.

Methods. The detailed procedure for the microbiological assay method employed has been given elsewhere.[2] Briefly, it consists of employing a pantothenate-free assay medium in which *Proteus morganii* will not grow. Addition of calcium pantothenate (Merck) in concentrations above 0.0001 μg per ml of medium permits growth of the test organism. Within limits the amount of growth is proportional to the pantothenate concentration. The increase in growth can be easily measured by employing a turbidimetric method. By plotting the turbidity values obtained against the concentration of pantothenate used, a "standard curve" is obtained.

To determine the pantothenic acid content of a specimen, an aqueous extract is prepared and autoclaved, and then a known quantity of this is incorporated in the assay medium. After inoculation with the test organism, the medium is incubated for 24 hours at 37°C and the turbidity is measured. Reference is then made to

* We wish to thank Merck & Company, Inc., for generously supplying the synthetic calcium pantothenate employed in this study.

1 Pelczar, M. J., Jr., and Porter, J. R., *J. Bact.*, 1941, **41**, 23.

2 Pelczar, M. J., Jr., and Porter, J. R., *J. Biol. Chem.*, 1941, **139**, 111.